DEVELOPMENT OF PROTOCOL IN THE IDENTIFICATION AND CULTIVATION OF INDIAN MEDICINAL LEECHES AND THE VIABILITY OF USE OF MEDICINAL LEECHES AS A SURGICAL TOOL

National Institute of Ayurveda,

Deemed To Be University (De-Novo)

Jaipur, Rajasthan

PROPOSED SYNOPSIS

FOR THE DEGREE OF

***Āyurveda Vidyāvāridhī* (Ph.D.)**

Scholar

Dr Anoop Sachi

M. S. (Ay.) Ph.D. Scholar

Supervisor

Prof. (Dr) Ashok Kumar.

M.S(Ay.), Ph.D. (Ay.)

Professor

Department of Shalya Tantra

National Institute of Ayurveda, Deemed To Be University (De-Novo)

Jorawar Singh Gate, Amer Road, Jaipur, Rajasthan – 302002

APPLICATION OF DISSERTATION

To

The Registrar

National institute of Ayurveda

Deemed to be University (De-novo), Jaipur

(Rajasthan)Through: - Proper Channel

Subject: -Submission of the Plan of dissertation / Protocol for the award of *Āyurveda Vidyāvāridhī* (Ph.D.) in the specialty of Shalya Tantra

Respected Sir,

With due regards, I am here by submitting the Protocol of my research project for the award of *ĀyurvedaVidyāvāridhī* (Ph.D.) in the specialty of Shalya Tantra. The particulars are given below**:**

Name of the Scholar	Dr. Anoop Sachi
Father's Name	N. Savyasachi
Date of Birth	07-05-1985
Permanent address	Revathi, Poothakulam P.O. Kollam, Kerala – 691302
Enrollment No.	NIA22030004
Previous exam passed	M.S. (Shalya Tantra)
Institution	National Institute of Ayurveda, deemed to be University (De-Novo), Jaipur (Rajasthan)
Language of the Thesis	English, Sanskrit
Title	**Development of protocol in the identification and cultivation of Indian Medicinal Leeches and the viability of use of Medicinal leeches as a Surgical tool**
Department	Shalya Tantra

Scholar **Supervisor**

Dr Anoop Sachi **Prof. (Dr) Ashok Kumar, M.S(Ay.), Ph.D. (Ay.)**

M. S. (Ay.) Ph.D. Scholar **Professor**

Department of Shalya Tantra

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INTRODUCTION

Medicinal leeches in Ayurveda

Bloodletting by means of *Jalouka* (leech) is called *Jaloukavacharana* in Ayurveda literature. *Jalouka* is considered the best among the *Sastras* (surgical instruments) and *Anusastras* (para surgical instruments) following *Kshara* and *Agni.* It has been used to eliminate *Pitta Pradushta Rakta* from the body for purpose of samshodhana as well as management of the certain disease conditions. [(1)] *Jaloukavacharana* is applied by Ayurvedic practitioners in the management of various dermatological conditions [(2)], chronic non-healing ulcers [(2)], deep vein thrombosis [(3)], thrombosed pile mass [(4)], Sub conjunctival haemorrhage [(5)], cases of peripheral arterial occlusion [(2)] and certain musculoskeletal diseases [(2)]. As per the major ayurvedic treatise, there are 6 varieties of leeches which can be used for the purpose of *raktamokshana* [(6)]. Ideal habitat and season for medicinal leech collection is mentioned by *Susruta*, followed by its preservation and cleansing before application. The post leech therapy procedures are also mentioned in detail in Susruta Samhita.

History of Hirudotherapy

Hirudotherapy or Medicinal leech therapy is one of the oldest treatment modalities used in almost all parts of the world. Its applicability ranges from various dermatological conditions, peripheral vascular conditions to musculoskeletal diseases. Its use was once upon a time so abundant in the continent of Europe that its excessive use led to depletion of leech population in Europe. With the advent of theory of infections in the causation of diseases, the medicinal leech therapy had a significant decline in the contemporary medical science in Europe. [(7)] But its use is still in practice amongst traditional practitioners all over the globe. The reintroduction of medicinal leech for bloodletting in the contemporary medicine happened following the studies done by the surgeons M. Derganc and F. Zdravic, who published an article describing its use to assist in tissue flap surgery after pioneering its successful application. [(8)] [(9)] Bloodletting by Medicinal leeches helps to prevent venous congestion and seroma formation of the flap thus

declining the rejection of flap and graft.

Medicinal leeches in Modern surgery

Medicinal leeches are used in modern surgery as a means for venous decongestion following reconstructive surgery. The idea behind it is the principle that a wound that continuously bleeds prevents venous congestion, thus increasing the take rate of the flap. The article published by surgeons M. Derganc and F. Zdravic, reported 20 cases of flaps with marked venous congestion managed with leeches, of which 70% had complete restoration of circulation and 30% had partial improvement. [9] One recent meta-analysis evaluating the outcome of leech therapy on the venous congestion of flaps and replanted appendages, demonstrated overall success rate of 77.8% and only 22% of them, ultimately requiring resection. [10] Since due to the report of cases of Aeromonas infection that results as a complication of leech therapy and increase in incidence of Cephalosporin resistant varieties, alternative means such as mechanical leeches have been explored by modern scientific community.

Current problems in leech therapy

Due to the destruction of the natural habitats of leeches and over exploitation of leech resources, there is a scarcity in the availability of Medicinal leeches. Due to contamination of leech sources by biological wastes and increased human contact with leech habitats, the safety use of the leeches that is collected from wild regions is still a major concern.

NEED OF THE STUDY

Identification of Medicinal leech species in India: Medicinal leeches used for medicinal leech therapy generally belong to the genera *Dinobdella*, *Hirudinaria*, *Hirudo*, *Limnatis*, *Myxobdella* and *Whitmania*. [11] Though the medicinal leeches belong to the above-mentioned genera, there is a lack of proper compiled literature mentioning its geographical distribution. The most common species found in India is said to be *Hirudinaria granulosa*. Literary evidence regarding its distribution in India is still not refined. Apart from *Hirudinaria granulosa*, medicinal leech species such as *Hirudinaria bpling, Asiaticobdella birmanica, Hirudinaria javanica, Hirudinaria manillensis* etc have been identified in India. [11] [12] [13] To be precise, the photographic images and geographical distribution of the medicinal leeches is not available in public domain for the medical community. This also prevents the thorough documentation of the peculiarities of the *jaloukavachara* by each species.

Classification of Medicinal leech species: *Acharya Sushruta* pioneered the classification of the medicinal leech species and non-medicinal leeches based on its morphological peculiarities. But the modern correlation of the 12 varieties of leeches has not yet been successfully done. [6] The only criteria that have been followed in practice for the identification of the medicinal leech species are the olive colour or brownish colour of the organism and its vermiform locomotion. But there are several leech species which possess colours other than above, say for example, black variety of *Hirudo Verbana* successfully employed in medicinal leech therapy.

Standard protocol for leech cultivation: Globally, the medicinal leech species are under threat and has been recognized as an endangered species. [14] Cause of this ranges from excessive use from the wild for medicinal purposes, abundance of harmful pesticides and fertilizers in its natural habitat, destruction of the water bodies which are its natural habitat and negative impact of the global climate change on medicinal leeches. The leeches collected from the wild do possess the risk of transmission of harmful pathogens. These factors point out to the importance of collected medicinal species, its preservation and its scientific cultivation. The extreme hot weather in the states north of India also pose a challenge to the other traditional leech cultivation methods. Though medicinal leech therapy is practiced widely throughout India, we do not have a scientific standardized leech cultivation center unlike many western countries. Many nations where medicinal leeches are used for traditional bloodletting therapy, have brought in strict regulations to ensure the safety of the procedure. For example, in Turkey, only leeches collected from certified farming centers can be used for the purpose of medicinal leech therapy.

Screening for the presence of ectoparasites and endoparasites in the medicinal leeches: The leech gut is known for the presence of symbiotic bacteria Aeromonas genospecies which have been suspected for several iatrogenic infections caused following leech therapy. [8] Apart from this, since natural leech habitat is not a sterile and probable known habitat for several microorganisms, leech body is known to harbor several endoparasites and ectoparasites which include several opportunistic bacteria and fungi. [15] Presence of virus and protozoa were also seen in some studies conducted on leeches. [16]

Cleansing of the leeches: Since leech therapy is an invasive procedure, each leech should be considered as a non-sterile syringe. Though the complete sterilization of live leeches is not possible, there is a need of suitable cleansing procedure to attain maximum sterility of medicinal leeches prior to its application. Though the use of *Haridra* and *Sarshapa kalka* is mentioned in the Ayurvedic treatise, study regarding its effect is not yet explored. [6] Study on the effect of application of *Haridra* proved in reducing some microbial load in the *Jalouka* collected from the wild. There have been a couple studies conducted towards find a proper cleansing method for leech which have met with some success. But the sucking quality of the leech were affected in some cases.

The unavailability of knowledge regarding the species of available medicinal leech in India, lack of adequate scientific leech cultivation centers, absence of agencies to certify such leech cultivation centers and no studies regarding the efficacy of leech cleansing techniques. The rise in *Rakta pradushta vikaras* due to unhealthy practices of the general population, the necessity of traditional leech therapy is still on rise. Thus, providing a solution to the above problems is the need the hour. This study tends to clear the path towards that goal.

LITERATURE REVIEW

Literature will be collected related to *Jalouka* (Medicinal leeches) from various authentic books, articles and others sources.

AIM & OBJECTIVES

Aim:

- To develop standardized protocols for identification, cultivation, screening & cleansing procedure for medicinal variety of leech prior to its therapeutic intervention.

Objective

- To identify the medicinal leech varieties used for *Jaloukavacharana* in India
- To classify the leech varieties based on the classification available in Ayurveda treatise.
- To develop a standard protocol for cultivation of medicinal leech.
- To screen the medicinal leeches for endoparasites and ectoparasites before medicinal use.

- To screen the medicinal leeches for endoparasites and ectoparasites before medicinal use after the classical cleansing procedures.

MATERIALS AND METHODS

Study Design

Basic/fundamental research

Developmental research

Experimental research

Animal Species – Medicinal Leech Available in India

Gender – Not Applicable

Procurement – Across India

Study Place – NIA, Jaipur

Age – Mature leech species

Weight Range – As per standard Protocol of CPCSEA guideline

Size – Average size according to weight range

Housing and Acclimatization – Standard international guidelines will be followed. Leeches will be kept at standard condition around 28°C under a light regime of 9.5D:14.5L

Study Duration

Literature research	–	1 month
Collection	–	2 Months
Identification	–	1 month
Classification	–	1 month
Cultivation	–	1 Year
Microbiological Study	–	2 months

Sample Size

Taxonomy Classification	–	200 *Jalouka*
Leech Cultivation	–	32 *Jalouka*
Cleansing	–	minimum 10 *Jalouka*
Microbiology Study	–	minimum of 5 *Jalouka* in each sample

Euthanasia

Euthanasia and appropriate disposal of experimental animals will be done as per standard

ethical guidelines in parallel with PCA act

Follow- Up

Animals will be sacrificed for Micro-biological study

Requirement

Well-equipped micrology lab and animal house.

Study Plan

Thorough literary research will be done for the collection of the information regarding different leech species used for medicinal leech therapy in India including their photographic images. Attempts will be made to collect the live leech varieties belong to each species. The leeches will be collected from different authentic leech vendors across India. Attempts would be made to collect leeches to the sources of supply to some of major Ayurvedic institutions and hospitals in India. The collected leech species will be identified and will then be attempted to be classified as per Ayurvedic nomenclature according to the morphological features mentioned in *Sushruta Samhita*. Data collection regarding methods of leech cultivation is to collected from different literature and several leech cultivations centers and an ideal leech cultivation unit suitable as per the climate of Jaipur will be setup. The lifecycle and the feeding habits of the preserved leeches will be observed. A healthy mature freshly cultured Medicinal leech sample will be subjected for the screening of ectoparasites and endoparasites. After assessing the ectoparasites and endoparasites, cleansing of the leeches will be done with *Sarshapa* and *Haridra kalka* and a sample of this leech group will again be screened separately for the presence of ectoparasites and endoparasites. The rest of the leeches will be observed for its sucking ability.

Study Subjects

Live Medicinal leech specimen collected from different parts of the country.

SELECTION CRITERIA

Inclusion Criteria

- Leech varieties that are known to be used for medicinal uses.
- Leech will be collected from authenticate source.
- Leech species with similar morphological features of *Nirvisha Jalouka* mentioned as per *Susruta Samhita.*

Exclusion Criteria

- Leech species having features similar to that of *Savisha Jalouka* mentioned as per *Susruta Samhita*.
- Leeches that are not hematophagous.
- Duplication source.

PROCEDURE

Procedure for collection and identification of the Medicinal leech

Medicinal leeches will be collected from authentic sources that have been consistently reliable in their supply of leeches to major reputed Ayurvedic institutions. Attempts will be made collect at least 10 leech sample from a major Ayurvedic institution in the Northern, Southern, North-eastern, North-western and Central parts of India. Leeches will also be collected from authentic leech cultivation centers known for its standards. At least 200 leech samples will be collected in this process and these leeches will be grouped according to their morphological classification. Attempts will be made identification based on binomial nomenclature. The leeches which could not be identified will be send to Zoological Survey of India Headquarters for its taxonomical classification.

Procedure for classification of Medicinal leeches as per the Ayurvedic Nomenclature

After assessing the species of the collected leech population, they are grouped as per the binomial nomenclature according to the International Code of Zoological Nomenclature. These species are then matched for the descriptions mentioned for the *Nirvisha Jalouka* mentioned in *Sushruta Samhita*. These will be classified as per the Ayurvedic nomenclature. If any variety *Jalouka* mentioned by *Sushruta*, does not find match in the collected specimens, thorough search in literature will be made to find such Medicinal leech to match such description. If any leeches collected did not match the description made by *Sushruta,* those will be separately mentioned in the study with their near possible correlation.

Procedure for cultivation of Medicinal leeches

Material Required:

- Adult Medicinal leeches will be purchased from different leech vendors all over India.
- Type of water: RO (reverse osmosis) water or dechlorinated water
- pH indicator paper – to measure the pH value of water.
- TDS meter – Digital TDS meter if required

- Urinometer – to measure specific gravity of water.
- Leech culture jars of approximate size (Minimum 500ml water for each *Jalouka*) to hold the individual leeches and for mating purpose.
- Bio-ceramic fragments will be placed in leech jars to avoid pH fluctuations and changes in biological properties of water.
- Earthen tanks of appropriate size to prevent extreme rise in temperature.

Total of 32 leeches will be selected to observe the leeches for study of cultivation. To adapt them to experimental conditions, each individual will be placed in a glass jar containing 0.5 l of dechlorinated water for one month. Later 16 among them will be selected with a pair of leeches belonging to the same species. To induce mating behavior, one leech will be paired with another in a single jar. In total, eight experimental jars will be prepared. Rest of the other 16 leeches will be placed in a container containing 6 liters of water for one month to maximize opportunities for copulation and fertilization. After that, specimens will be kept individually in moist moss-filled containers for cocoon deposition. Cocoons will be collected and kept separately on wet moss. Hatchlings hatched on the same day will be selected for the life-history survey.

Procedure for screening for ectoparasites and endoparasites in the Medicinal leeches

A sample of minimum 5 leeches will be selected from the leeches that are cultured separately as per their species. If a minimum of 20 leech is not available in a group, the screening of that group of leeches will be dropped from the study. These leeches will be subjected to the screening for the presence of ectoparasites and endoparasites. Water samples will be collected from the glass jar in which these leeches are cultured and they will be sent to the laboratory for screening for the presence of microorganisms. Samples will then be collected from leech body and leech mouth & anus separately, which will be pooled and also be send for screening.

To study the endoparasites, five leeches in each group will be selected and dissected. Three dissected jaws and one pharynx from each leech will be collected into one test-tube. The jaws/pharynx and intestine will be separately collected. These samples will be pooled and sent to laboratory for screening.

Screening will be done on the basis of Microbial culture and Microbial load analysis.

Procedure for Cleansing procedure of the leeches

Materials required:

- Cleansing agent
  - *Sarshapa kalka* & *Haridra kalka* – Q.S

As per literature, the leeches will be treated with *Sarshapa* and *Haridra kalka* for 45 min followed by rinsing with RO water.

PREVIOUS WORK

Identification of Medicinal Leeches

1. Phull G, Dhule M, Phull R, Gupta M. Identification of a New Species of the Medicinal Leeches in India using DNA Barcoding Technique - a Breakthrough. JPRI [Internet]. 7Jun.2021 [cited 19Nov.2022];33 (30B):191-9. Available from: https://journaljpri.com/index.php/JPRI/article/view/2484.
2. Pallabi Pathak, Saidul Islam, Joken Bam, Debasish Bhattacharya, Manoranjan Das, Dhruba Jyoti Kalita, Girin Kumar Saikia, Gouranga Das, Gautam Bordoloi. Distribution of aquatic and terrestrial leeches in Assam, India. J Entomol Zool Stud 2020;8(4):605-609.

Cultivation of medicinal leeches

1. Mahesh et al. Natural water habitat is best for leech farming: An observational pilot study. World Journal of Pharmacy and Pharmaceutical Sciences, 2018: 7(8), 1037-1045. doi: 10.20959/wjpps20188-12136
2. Malek, M., Jafarifar, F., Roohi Aminjan, A., Salehi, H., & Parsa, H. Culture of a new medicinal leech: growth, survival and reproduction of Hirudo orientalis Utevsky and Trontelj, 2005 under laboratory conditions. Journal of Natural History,2019: 53(11-12), 627–637. doi:10.1080/00222933.2019.1597200
3. Kumar M, Rani M. Leech farming in natural habitat: An observational report. AYUHOM. Jan.2020;7(1):15-19. doi: 10.4103/AYUHOM.AYUHOM_18_21.

Screening for ectoparasites and endoparasites

1. Nehili, M., Ilk, C., Mehlhorn, H., Ruhnau, K., Dick, W., & Njayou, M. Experiments on the possible role of leeches as vectors of animal and human pathogens: a light and electron microscopy study. Parasitology Research, 1994;80(4), 277–290. doi:10.1007/bf02351867

2. Nelson MC, Graf J. Bacterial symbioses of the medicinal leech Hirudo verbana. Gut Microbes. 2012;3(4):322-331. doi:10.4161/gmic.20227
3. Derebail, Gururaja. Evaluation of organisms present in the gut of Hirudinaria granulosa or Indian cattle leech used for the purpose of Hirudotherapy (Jalukavacharana) in Ayurveda -A Metagenomic study. J Ayurveda Integr Med Sci 2018;6:56-61. doi:10.21760/jaims.3.6.9

Cleansing procedure for Medicinal leeches

1. Giri RV, Tantry DG, Shetty P et.al. Evaluation of the effect of haridra (turmeric) on leech gut flora: a metagenomics study. Int J Health Sci Res. 2019; 9(8):229-238.
2. Hokelek, M., Güneren, E., & Eroglu, C. An experimental study to sterilize medicinal leeches. European Journal of Plastic Surgery.2002; 25(2), 81–85. doi:10.1007/s00238-002-0348-x
3. Aydin, Atakan et al. External decontamination of wild leeches with hypochloric acid. BMC infectious diseases.2004: 28(4), doi:10.1186/1471-2334-4-28

REFERENCES

1. Acharya JT, Kavyatirtha NRA, editors. Susruta Samhita of Susruta (Nibandhasangraha, Dalhanacharya,comme,Sanskrit) Varanasi: Chaukhamba Surbharati Prakashan; 2017;p.45.11/3.
2. Bhardwaj R, Tanwar AK, Gupta HC. A Conceptual Review of Jalaukavacharana. International Journal of Ayurveda and Pharma Research. 2019 Jan; 7(1): 54-59.
3. Kaur , Shailly C, Singh A. Role of Ayurvedic Medicine and Leech therapy in management of Deep Vein Thrombosis : A Case Study. Journal of Biologic & Scientific Opinion. 2015 Apr; 3(2): 87-90.
4. Bhagat PJ RSLA. Clinical efficacy of Jalaukawacharana (leech application) in Thrombosed piles. Ayu. 2012 Apr; 33(2): 261-263.
5. Sreenath PS GB. Clinical efficacy of Jaloukavacharana (Leech Therapy) in Sub Conjunctival Haemorrhage - A Case Study. J Ayurveda Integr Med Sci [Internet]. 2021 Nov 7; 6(5): 299-302. Available from: https://www.jaims.in/jaims/article/view/1504.
6. Acharya JT, Kavyatirtha NRA, editors. Susrutasamhita of Susruta (Nibandhasangraha, Dalhanacharya,comme,Sanskrit) Varanasi: Chaukhamba Surbharati Prakashan; 2017.p.55-57.13/3-19.
7. Whitaker IS, Rao J, Izadi D, Butler PE. Hirudomedicinalis: ancient origins of, and trends in the use of medicinalleeches throughout history. British Journal of Oral and Maxillofacial Surgery. 2004 Apr 1; 42(2): 133-137.
8. Whitaker IS, Izadi D, Oliver DW, Monteath G, Butler PE. Hirudo Medicinalis and the plastic surgeon. The British Association of Plastic Surgeons. 2004 July; 57(4): 348-353.

9. Derganc M, Zdravic F. Venous congestion of flap treated by application of leeches. Br J Plast Surg. 1960; 13: 187-192.
10. Whitaker IS, Oboumarzouk O, Rozen MW, Naderi N, Balasubramanian SP, Azzopardi EA, et al. The efficacy of medicinal leeches in plastic and reconstructive surgery: A systematic review of 277 reported clinical cases. Microsurgery. 2012 Mar 8; 32(3): 240–250.
11. Phull G DMPRGM. Identification of a New Species of the Medicinal Leeches in India using DNA Barcoding Technique - a Breakthrough. JPRI [Internet]. 2021 Jun 7; 33(30B): 191-199.
12. Lone H, Ahmad T, Anwar M, Habib S, Sofi G, Imam H. Leech Therapy- A Holistic Approach of Treatment in Unani (Greeko-Arab) Medicine. Anc Sci Life. 2011 Jul; 31(1): 31-35.
13. Pathak P, Islam S, Bam J, Bhattacharya D, Das M, Kalita DJ, et al. Distribution of aquatic and terrestrial leeches in. Journal of Entomology and Zoology Studies. 2020; 8(4): 605-609.
14. Collier KJ, Probert PK, Jeffries M. Conservation of aquatic invertebrates: concerns, challenges and conundrums. Aquatic Conserv: Mar. Freshw. Ecosyst. 2016; 26: 817– 837.
15. Litwinowicz A, Blaszkowska J. Hirudo verbana is a source of fungal isolates potentially. African Journal of Microbiology Research. 2013 November 28; 7(47): 5358-5363.
16. Nehili M, Ilk C, Mehlhorn H, Ruhnau K, Dick W, Njayou M. Experiments on the possible role of leeches as vectors of animal. Parasitol Res. 1994; 80(4): 277-290.
17. Abdualkader AM, Ghawi AM, Alaama A, Awang M, Merzouk A. Leech therapeutic applications. Indian J Pharm Sci. 2013 March-April; 75(2): 127-137.
18. Dergan M, Zdravic F. Venous congestion of flaps treated by application of leeches. British Journal of Plastic Surgery. 1960 July; 13(1): 187-192.

PROPOSED CONTENT OF DISSERTATION: -

All the observations recorded and results obtained will be computerized statistically to draw various conclusions. The project will be presented in the form of a thesis with following contents-

- Acknowledgement
- Introduction
- Re-view of literature
  - Classical review
  - Modern review
- Materials and Methods
- Observations and Results
- Discussions and Conclusion
- Summary
- Bibliography
- Appendix

Scholar

Dr. Anoop Sachi.

M.S.(Ay)

Ph.D. Scholar

Supervisor

Prof. (Dr). Ashok Kumar.

M.S.(Ay), Ph.D. (Ay)

Professor

Department of Shalya Tantra

National Institute of Ayurveda, Deemed To Be University (De-Novo)

Jorawar Singh Gate, Amer Road, Jaipur, Rajasthan – 302002